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COMPOSITIONS ISOLATED FROM STROMAL CELLS AND METHODS FOR THEIR USE

Technical Field of the Invention

This invention relates to polynucleotides and polypeptides derived from lymph node stromal cells from flaky skin (*fsn* -/-) mice and their use in therapeutic methods.

Background of the Invention

Lymph vessels and nodes are important components of the body's immune system. Lymph nodes are small lymphatic organs that are located in the path of lymph vessels. Large molecules and cells, including foreign substances, enter into the lymphatic vessels and, in circulating through these vessels, pass through the lymph nodes. Here, any foreign substances are concentrated and exposed to lymphocytes. This triggers a cascade of events that constitute an immune response, protecting the body from infection and from cancer.

Lymph nodes are surrounded by a dense connective tissue network that forms a supporting capsule. This network extends into the body of the lymph node, forming an additional framework of support. Throughout the remainder of the organ, a fine meshwork can be identified that comprises reticular fibres and the reticular cells that produce and surround the fibres. These features provide a support for the main functional cells of the lymphatic system, which are T- and B-lymphocytes. Additional cell types found in lymph nodes include macrophages, follicular dendritic cells, and endothelial cells that line the blood vessels servicing the node.

The cells within lymph nodes communicate with each other in order to defend the body against foreign substances. When a foreign substance, or antigen, is present, it is detected by macrophages and follicular dendritic cells that take up and process the antigen, and display parts of it on their cell surface. These cell surface antigens are then presented to T- and B-lymphocytes, causing them to proliferate and differentiate into

activated T-lymphocytes and plasma cells, respectively. These cells are released into the circulation in order to seek out and destroy antigen. Some T- and B-lymphocytes will also differentiate into memory cells. Should these cells come across the same antigen at a later date, the immune response will be more rapid.

Once activated T- and B-lymphocytes are released into the circulation, they can perform a variety of functions that lead to the eventual destruction of antigen. Activated T-lymphocytes can differentiate into cytotoxic lymphocytes (also known as killer T-cells) which recognise other cells that have foreign antigens on their surface and kill the cell by causing them to lyse. Activated T-lymphocytes can also differentiate into helper T-cells which will then secrete proteins in order to stimulate B-lymphocytes, and other T-lymphocytes, to respond to antigens. In addition, activated T-lymphocytes can differentiate into suppressor T-cells which secrete factors that suppress the activity of B-lymphocytes. Activated B-lymphocytes differentiate into plasma cells, which synthesise and secrete antibodies that bind to foreign antigens. The antibody-antigen complex is then detected and destroyed by macrophages, or by a group of blood constituents known as complement.

Lymph nodes can be dissociated and the resulting cells grown in culture. Cells that adhere to the tissue culture dishes can be maintained for some length of time and are known as stromal cells. The cultured cells are a heterogeneous population and can be made up of most cells residing within lymph nodes, such as reticular cells, follicular dendritic cells, macrophages and endothelial cells. It is well known that bone marrow stromal cells play a critical role in homing, growth and differentiation of hematopoietic progenitor cells. Proteins produced by stromal cells are necessary for the maintenance of plasma cells *in vitro*. Furthermore, stromal cells are known to secrete factors and present membrane-bound receptors that are necessary for the survival of lymphoma cells.

An autosomal recessive mutation, designated flaky skin (*fsn* -/-), has been described in the inbred A/J mouse strain (The Jackson Laboratory, Bar Harbour, ME). The mice have a skin disorder similar to psoriasis in humans. Psoriasis is a common disease affecting 2% of the population, which is characterised by a chronic inflammation

associated with thickening and scaling of the skin. Histology of skin lesions shows increased proliferation of the cells in the epidermis, the uppermost layer of skin, together with the abnormal presence of inflammatory cells, including lymphocytes, in the dermis, the layer of skin below the epidermis. While the cause of the disease is unclear, psoriasis is associated with a disturbance of the immune system involving T lymphocytes. The disease occurs more frequently in family members, indicating the involvement of a genetic factor as well. Mice with the *fsn* gene mutation have not only a psoriatic-like skin disease but also other abnormalities involving cells of the immune and hematopoietic system. These mice have markedly increased numbers of lymphocytes associated with enlarged lymphoid organs, including the spleen and lymph nodes. In addition, their livers are enlarged, and the mice are anaemic. Genes and proteins expressed in abnormal lymph nodes of *fsn-/-* mice may thus influence the development or function of cells of the immune and hematopoietic system, the response of these cells in inflammatory disorders, and the responses of skin and other connective tissue cells to inflammatory signals.

There is a need in the art to identify genes encoding proteins that function to modulate all cells of the immune system. These proteins from normal or abnormal lymph nodes may be useful in modifying the immune responses to tumour cells or infectious agents such as bacteria, viruses, protozoa and worms. Such proteins may be useful in the treatment of disorders where the immune system initiates unfavourable reactions to the body, including Type I hypersensitivity reactions (such as hay fever, eczema, allergic rhinitis and asthma), and Type II hypersensitivity reactions (such as transfusion reactions and haemolytic disease of newborns). Other unfavourable reactions are initiated during Type III reactions, which are due to immune complexes forming in infected organs during persistent infection or in the lungs following repeated inhalation of materials from moulds, plants or animals, and in Type IV reactions in diseases such as leprosy, schistosomiasis and dermatitis.

Novel proteins of the immune system may also be useful in treating autoimmune diseases where the body recognises itself as foreign. Examples of such diseases include

rheumatoid arthritis, Addison's disease, ulcerative colitis, dermatomyositis and lupus. Such proteins may also be useful during tissue transplantation, where the body will often recognise the transplanted tissue as foreign and attempt to kill it, and also in bone marrow transplantation when there is a high risk of graft-versus-host disease where the transplanted cells attack their host cells, often causing death.

There thus remains a need in the art for the identification and isolation of genes encoding proteins expressed in cells of the immune system for use in the development of therapeutic agents for the treatment of disorders including those associated with the immune system.

Summary of the Invention

The present invention provides polypeptides and functional portions of polypeptides expressed in lymph node stromal cells of *fsn* -/- mice, together with polynucleotides encoding such polypeptides, expression vectors and host cells comprising such polynucleotides, and methods for their use.

In specific embodiments, isolated polypeptides are provided that comprise an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 11-20, 30-38, 47-53 and 59-61, and variants of such sequences, as defined herein. Isolated polypeptides which comprise at least a functional portion of a polypeptide comprising an amino acid sequence selected from the group consisting of: (a) sequences provided in SEQ ID NO: 11-20, 30-38, 47-53 and 59-61; and (b) variants of a sequence of SEQ ID NO: 11-20, 30-38, 47-53 and 59-61, as defined herein, are also provided.

In other embodiments, the present invention provides isolated polynucleotides comprising a nucleotide sequence selected from the group consisting of: (a) sequences provided in SEQ ID NO: 1-10, 21-29, 39-46 and 58; (b) complements of sequences provided in SEQ ID NO: 1-10, 21-29, 39-46 and 58; (c) reverse complements of sequences provided in SEQ ID NO: 1-10, 21-29, 39-46 and 58; (d) reverse sequences of

sequences provided in SEQ ID NO: 1-10, 21-29, 39-46 and 58; and (e) variants of the sequences of (a) – (d), as defined herein.

In related embodiments, the present invention provides expression vectors comprising the above polynucleotides, together with host cells transformed with such vectors.

As detailed below, the isolated polynucleotides and polypeptides of the present invention may be usefully employed in the preparation of therapeutic agents for the treatment of immunological disorders.

In related embodiments, methods for modulating the growth of blood vessels, and for the treatment of disorders such as inflammatory disorders, disorders of the immune system, cancer, tumour-necrosis factor-mediated disorders, and viral disorders are provided. Examples of such disorders include HIV-infection; epithelial, lymphoid, myeloid, stromal and neuronal cancers; arthritis; inflammatory bowel disease; and cardiac failure.

The above-mentioned and additional features of the present invention, together with the manner of obtaining them, will be best understood by reference to the following more detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Figure 1 is the amino acid sequence of the murine FGF receptor muFGFβ (SEQ ID NO: 31), showing the location of the transmembrane domain, the Ig domain, splice sites, and putative glycosylation and SHP binding sites. Specifically, the underlined regions represent the signal peptide, IgG1 domain, IgG2 domain and transmembrane domain; the regions in bold italics and underlined represent the four putative glycosylation sites; and the bold underlined regions represent the two putative SHP-2 binding sites.

Figure 2A shows the induction of genes under the control of the SRE. NIH-3T3 SRE cells were stimulated with a titration of FGF-2 in the presence of 10 μg/ml of

heparin for 6 hours. Closed circles represent media alone, open squares represent titration of FGF-2. Figure 2B shows the competition analysis of NIH-3T3 SRE cells treated with a standard dose of FGF-2 + heparin in the presence of increasing concentrations of FGFR2Fc (closed diamonds), FGFR5βFc (closed squares) , FGFR5γFc (closed triangles) and FGF-2 alone (asterisk). The mean and SD were calculated for both experiments from 3 separate wells and are represented as fold-induction of the reporter gene relative to .

Figure 3 illustrates the stimulation of growth of RAW264.10 cells by FGFR5β and FGFR5γ. This stimulation was not observed when FGF-2 and FGFR2 were used as controls. This stimulation was also not induced by the growth medium.

Figure 4 illustrates the enhancing proliferative effect of FGFR5β and FGFR5γ on PHA-induced PBMC. The enhanced proliferation was not observed when FGFR2 or purified IgG Fc was used.

Figure 5 shows the enhanced proliferation of anti-CD3 stimulated PBMC by FGFR5β and FGFR5γ. The enhanced proliferation was not observed when FGFR2 or purified FC was used as stimulants.

Figure 6 demonstrates that FGFR5β and FGFR5γ, or the controls FGFR2 or IgG Fc did not stimulate proliferation of PBMC in the absence of PHA.

Figure 7 illustrates the stimulation of PBMC adherence by FGFR5β and FGFR5γ but not by FGFR2 or purified IgG Fc.

Figure 8 shows the stimulation of adherent PHA-stimulated PBMC by FGFR5β and FGFR5γ but not by purified IgG Fc.

Figure 9 illustrates the stimulation of NK cell adherence by FGFR5β and FGFR5γ as measured by the presence of anti-CD56 antibodies, markers of NK cells. The filled histograms represent the adherent PBMC stained with the NK cell marker CD56 and the open histograms represent the same cells stained with the isotype-matched control antibody.

Detailed Description of the Invention

In one aspect, the present invention provides polynucleotides isolated from lymph node stromal cells of *fsn* -/- mice and isolated polypeptides encoded by such polynucleotides.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments. Anti-sense polynucleotides and techniques involving anti-sense polynucleotides are well known in the art and are described, for example, in Robinson-Benion *et al.*, *Methods in Enzymol.* 254: 363-375, 1995 and Kawasaki *et al.*, *Artific. Organs* 20: 836-848, 1996.

In specific embodiments, the isolated polynucleotides of the present invention comprise a polynucleotide sequence selected from the group consisting of sequences provided in SEQ ID NO: 1-10, 21-29, 39-46 and 58.

Complements of such isolated polynucleotides, reverse complements of such isolated polynucleotides and reverse sequences of such isolated polynucleotides are also provided, together with polynucleotides comprising at least a specified number of contiguous residues (*x*-mers) of any of the above-mentioned polynucleotides, extended sequences corresponding to any of the above polynucleotides, antisense sequences corresponding to any of the above polynucleotides, and variants of any of the above polynucleotides, as that term is described in this specification.

The definitions of the terms "complement", "reverse complement" and "reverse sequence", as used herein, are best illustrated by the following example. For the sequence 5' AGGACC 3', the complement, reverse complement and reverse sequence are as follows:

complement	3' TCCTGG 5'
reverse complement	3' GGTCCT 5'
reverse sequence	5' CCAGGA 3'.

Some of the polynucleotides of the present invention are "partial" sequences, in that they do not represent a full length gene encoding a full length polypeptide. Such partial sequences may be extended by analyzing and sequencing various DNA libraries using primers and/or probes and well known hybridization and/or PCR techniques. Partial sequences may be extended until an open reading frame encoding a polypeptide, a full length polynucleotide and/or gene capable of expressing a polypeptide, or another useful portion of the genome is identified. Such extended sequences, including full length polynucleotides and genes, are described as "corresponding to" a sequence identified as one of the sequences of SEQ ID NO: 1-10, 21-29, 39-46 and 58, or a variant thereof, or a portion of one of the sequences of SEQ ID NO: 1-10, 21-29, 39-46 and 58, or a variant thereof, when the extended polynucleotide comprises an identified sequence or its variant, or an identified contiguous portion (*x*-mer) of one of the sequences of SEQ ID NO: 1-10, 21-29, 39-46 and 58, or a variant thereof. Such extended polynucleotides may have a length of from about 50 to about 4,000 nucleic acids or base pairs, and preferably have a length of less than about 4,000 nucleic acids or base pairs, more preferably yet a length of less than about 3,000 nucleic acids or base pairs, more preferably yet a length of less than about 2,000 nucleic acids or base pairs. Under some circumstances, extended polynucleotides of the present invention may have a length of less than about 1,800 nucleic acids or base pairs, preferably less than about 1,600 nucleic acids or base pairs, more preferably less than about 1,400 nucleic acids or base pairs, more preferably yet less than about 1,200 nucleic acids or base pairs, and most preferably less than about 1,000 nucleic acids or base pairs.

Similarly, RNA sequences, reverse sequences, complementary sequences, antisense sequences, and the like, corresponding to the polynucleotides of the present invention, may be routinely ascertained and obtained using the cDNA sequences identified as SEQ ID NO: 1-10, 21-29, 39-46 and 58.

The polynucleotides identified as SEQ ID NO: 1-10, 21-29, 39-46 and 58 contain open reading frames ("ORFs") or partial open reading frames encoding polypeptides or functional portions of polypeptides. Open reading frames may be identified using techniques that are well known in the art. These techniques include, for example, analysis for the location of known start and stop codons, most likely reading frame identification based on codon frequencies, etc. Open reading frames and portions of open reading frames may be identified in the polynucleotides of the present invention. Suitable tools and software for ORF analysis are available, for example, on the Internet at http://www.ncbi.nlm.nih.gov/gorf/gorf.html. Suitable tools and software for ORF analysis are also available through other distribution channels. Exemplary tools and software include, for example, GeneWise, available from The Sanger Center, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, United Kingdom; Diogenes, available from Computational Biology Centers, University of Minnesota, Academic Health Center, UMHG Box 43 Minneapolis MN 55455; and GRAIL, available from the Informatics Group, Oak Ridge National Laboratories, Oak Ridge, Tennessee TN. Once a partial open reading frame is identified, the polynucleotide may be extended in the area of the partial open reading frame using techniques that are well known in the art until the polynucleotide for the full open reading frame is identified. Thus, open reading frames encoding polypeptides and/or functional portions of polypeptides may be identified using the polynucleotides of the present invention.

Once open reading frames are identified in the polynucleotides of the present invention, the open reading frames may be isolated and/or synthesized. Expressible genetic constructs comprising the open reading frames and suitable promoters, initiators, terminators, etc., which are well known in the art, may then be constructed. Such genetic constructs may be introduced into a host cell to express the polypeptide encoded by the

open reading frame. Suitable host cells may include various prokaryotic and eukaryotic cells, including plant cells, mammalian cells, bacterial cells, algae and the like.

In another aspect, the present invention provides isolated polypeptides encoded, or partially encoded, by the above polynucleotides. The term "polypeptide", as used herein, encompasses amino acid chains of any length including full length proteins, wherein amino acid residues are linked by covalent peptide bonds. Polypeptides of the present invention may be naturally purified products, or may be produced partially or wholly using recombinant techniques. The term "polypeptide encoded by a polynucleotide" as used herein, includes polypeptides encoded by a nucleotide sequence which includes the partial isolated DNA sequences of the present invention. In specific embodiments, the inventive polypeptides comprise an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 11-20, 30-38, 47-53, 59 and variants of such sequences.

Polypeptides encoded by the polynucleotides of the present invention may be expressed and used in various assays to determine their biological activity. Such polypeptides may be used to raise antibodies, to isolate corresponding interacting proteins or other compounds, and to quantitatively determine levels of interacting proteins or other compounds.

All of the polynucleotides and polypeptides described herein are isolated and purified, as those terms are commonly used in the art. Preferably, the polypeptides and polynucleotides are at least about 80% pure, more preferably at least about 90% pure, and most preferably at least about 99% pure.

As used herein, the term "variant" comprehends nucleotide or amino acid sequences different from the specifically identified sequences, wherein one or more nucleotides or amino acid residues is deleted, substituted, or added. Variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant sequences (polynucleotide or polypeptide) preferably exhibit at least 50%, more preferably at least 75%, and most preferably at least 90% or 95% identity to a sequence of the present invention. The percentage identity is determined by aligning the two

sequences to be compared, determining the number of identical residues in the aligned portion, dividing that number by the total length of the inventive, or queried, sequence and multiplying the result by 100.

Polynucleotide or polypeptide sequences may be aligned, and percentage of identical residues in a specified region may be determined against another polynucleotide, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences are the BLASTN and FASTA algorithms. Polynucleotides may also be analyzed using the BLASTX algorithm, which compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database. The similarity of polypeptide sequences may be examined using the BLASTP or FASTX algorithms. Both the BLASTN and BLASTP software are available on the NCBI anonymous FTP server (ftp://ncbi.nlm.nih.gov) under /blast/executables/ and are available from the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894 USA. The BLASTN algorithm versions 2.0.6 [Sept-16-1998] and version 2.0.11 [Jan-20-2000], set to the default parameters described in the documentation and distributed with the algorithm, are preferred for use in the determination of variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN and BLASTP, is described at NCBI's website at URL http://www.ncbi.nlm.nih.gov/BLAST/newblast.html and in the publication of Altschul *et al.*, "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402, 1997.

The computer algorithm FASTA is available on the Internet at the ftp site ftp://ftp.virginia.edu/pub/fasta/. The FASTA software package is also available from the University of Virginia by contacting David Hudson, Assistant Provost for Research, University of Virginia, PO Box 9025, Charlottesville, VA 22906-9025. FASTA Version3.1t11, August 1998, set to the default parameters described in the documentation and distributed with the algorithm, is preferred for use in the determination of variants

according to the present invention. The use of the FASTA algorithm is described in Pearson and Lipman, "Improved Tools for Biological Sequence Analysis," *Proc. Natl. Acad. Sci. USA* 85:2444-2448, 1988 and Pearson, "Rapid and Sensitive Sequence Comparison with FASTP and FASTA," *Methods in Enzymol.* 183:63-98, 1990. The use of the FASTX algorithm is described in Pearson *et al.*, "Comparison of DNA sequences with protein sequences," *Genomics* 46:24-36, 1997.

The following running parameters are preferred for determination of polynucleotide alignments and similarities using BLASTN that contribute to the E values and percentage identity: Unix running command: blastall -p blastn -d embldb -e 10 -G 0 -E 0 -r 1 -v 30 -b 30 -i queryseq –o results; and parameters are as follows: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -r Reward for a nucleotide match (BLAST only) [Integer]; -v Number of one-line descriptions (V) [Integer]; -b Number of alignments to show (B) [Integer]; -i Query File [File In]; -o BLAST report Output File [File Out] Optional.

For BLASTP analyses of polypeptide sequences, the following running parameters are preferred: blastall –p blastp –d swissprotdb –e 10 –G 0 –E 0 –v 30 –b 30 –i queryseq –o results; and the parameters are as follows: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -v Number of one-line descriptions (v) [Integer]; -b Number of alignments to show (b) [Integer]; -I Query File [File In]; -o BLAST report Output File [File Out] Optional.

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

The percentage identity of a polynucleotide or polypeptide sequence is determined by aligning polynucleotide and polypeptide sequences using appropriate algorithms, such as BLASTN or BLASTP, respectively, set to default parameters; identifying the number of identical nucleic or amino acids over the aligned portions; dividing the number of identical nucleic or amino acids by the total number of nucleic or amino acids of the polynucleotide or polypeptide of the present invention; and then multiplying by 100 to determine the percentage similarity. By way of example, a queried polynucleotide having 220 nucleic acids has a hit to a polynucleotide sequence in the EMBL database having 520 nucleic acids over a stretch of 23 nucleotides in the alignment produced by the BLASTN algorithm using the default parameters. The 23 nucleotide hit includes 21 identical nucleotides, one gap and one different nucleotide. The percentage identity of the queried polynucleotide to the hit in the EMBL database is thus 21/220 times 100, or 9.5%. The similarity of polypeptide sequences may be determined in a similar fashion.

The BLASTN and FASTA algorithms also produce "Expect" values for alignments. The Expect value (E) indicates the number of hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database, such as the preferred EMBL database, indicates true similarity. For example, an E value of 0.1 assigned to a hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion of the sequence with a similar score simply by chance. By this criterion, the aligned and matched portions of the sequences then have a probability of 90% of being the same. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN or FASTA algorithm.

According to one embodiment, "variant" polynucleotides, with reference to each of the polynucleotides of the present invention, preferably comprise sequences having the same number or fewer nucleic acids than each of the polynucleotides of the present

invention and producing an E value of 0.01 or less when compared to the polynucleotide of the present invention. That is, a variant polynucleotide is any sequence that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or FASTA algorithms set at the default parameters. According to a preferred embodiment, a variant polynucleotide is a sequence having the same number or fewer nucleic acids than a polynucleotide of the present invention that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or FASTA algorithms set at the default parameters.

Alternatively, variant polynucleotide sequences hybridize to the recited polynucleotide sequence under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65 °C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65 °C.

The present invention also encompasses polynucleotides that differ from the disclosed sequences but that, as a consequence of the degeneracy of the genetic code, encode a polypeptide which is the same as that encoded by a polynucleotide of the present invention. Thus, polynucleotides comprising sequences that differ from the polynucleotide sequences provided in SEQ ID NO: 1-10, 21-29, 39-46 and 58, or complements, reverse sequences, or reverse complements thereof, as a result of conservative substitutions, are contemplated by and encompassed within the present invention. Additionally, polynucleotides comprising sequences that differ from the polynucleotide sequences provided in SEQ ID NO: 1-10, 21-29, 39-46 and 58, or complements, reverse complements or reverse sequences thereof, as a result of deletions and/or insertions totaling less than 10% of the total sequence length are also contemplated by and encompassed within the present invention. Similarly, polypeptides comprising sequences that differ from the polypeptide sequences provided in SEQ ID NO: 11-20, 30-38, 47-53 and 59-61, as a result of amino acid substitutions, insertions,

and/or deletions totaling less than 10% of the total sequence length are contemplated by and encompassed within the present invention.

Polynucleotides of the present invention also comprehend polynucleotides comprising at least a specified number of contiguous residues (*x*-mers) of any of the polynucleotides identified as SEQ ID NO: 1-10, 21-29, 39-46 and 58, complements, reverse sequences, and reverse complements of such sequences, and their variants. Similarly, polypeptides of the present invention comprehend polypeptides comprising at least a specified number of contiguous residues (*x*-mers) of any of the polypeptides identified as SEQ ID NO: 11-20, 30-38, 47-53 and 59-61, and their variants. As used herein, the term "*x*-mer," with reference to a specific value of "*x*," refers to a sequence comprising at least a specified number ("*x*") of contiguous residues of any of the polynucleotides identified as SEQ ID NO: 1-10, 21-29, 39-46 and 58, or the polypeptides identified as SEQ ID NO: 11-20, 30-38, 47-53 and 59-61. According to preferred embodiments, the value of *x* is preferably at least 20, more preferably at least 40, more preferably yet at least 60, and most preferably at least 80. Thus, polynucleotides and polypeptides of the present invention comprise a 20-mer, a 40-mer, a 60-mer, an 80-mer, a 100-mer, a 120-mer, a 150-mer, a 180-mer, a 220-mer, a 250-mer, a 300-mer, 400-mer, 500-mer or 600-mer of a polynucleotide or polypeptide identified as SEQ ID NO: 1-53, 58 and 59, and variants thereof.

The inventive polynucleotides may be isolated by high throughput sequencing of cDNA libraries prepared from lymph node stromal cells of *fsn* -/- mice as described below in Example 1. Alternatively, oligonucleotide probes based on the sequences provided in SEQ ID NO: 1-10, 21-29, 39-46 and 58 can be synthesized and used to identify positive clones in either cDNA or genomic DNA libraries from lymph node stromal cells of *fsn* -/- mice by means of hybridization or polymerase chain reaction (PCR) techniques. Probes can be shorter than the sequences provided herein but should be at least about 10, preferably at least about 15 and most preferably at least about 20 nucleotides in length. Hybridization and PCR techniques suitable for use with such oligonucleotide probes are well known in the art (see, for example, Mullis *et al.*, *Cold*

Spring Harbor Symp. Quant. Biol., 51:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989; Sambrook *et al.*, *Molecular cloning –a laboratory manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Positive clones may be analyzed by restriction enzyme digestion, DNA sequencing or the like.

The polynucleotides of the present invention may alternatively be synthesized using techniques that are well known in the art. The polynucleotides may be synthesized, for example, using automated oligonucleotide synthesizers (*e.g.*, Beckman Oligo 1000M DNA Synthesizer) to obtain polynucleotide segments of up to 50 or more nucleic acids. A plurality of such polynucleotide segments may then be ligated using standard DNA manipulation techniques that are well known in the art of molecular biology. One conventional and exemplary polynucleotide synthesis technique involves synthesis of a single stranded polynucleotide segment having, for example, 80 nucleic acids, and hybridizing that segment to a synthesized complementary 85 nucleic acid segment to produce a 5 nucleotide overhang. The next segment may then be synthesized in a similar fashion, with a 5 nucleotide overhang on the opposite strand. The "sticky" ends ensure proper ligation when the two portions are hybridized. In this way, a complete polynucleotide of the present invention may be synthesized entirely *in vitro*.

Polypeptides of the present invention may be produced recombinantly by inserting a DNA sequence that encodes the polypeptide into an expression vector and expressing the polypeptide in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, insect, yeast or a mammalian cell line such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring polypeptides, portions of naturally occurring polypeptides, or other variants thereof.

In a related aspect, polypeptides are provided that comprise at least a functional portion of a polypeptide having an amino acid sequence selected from the group

consisting of sequences provided in SEQ ID NO: 11-20, 30-38, 47-53 and 59-61 and variants thereof. As used herein, the "functional portion" of a polypeptide is that portion which contains the active site essential for affecting the function of the polypeptide, for example, the portion of the molecule that is capable of binding one or more reactants. The active site may be made up of separate portions present on one or more polypeptide chains and will generally exhibit high binding affinity. Such functional portions generally comprise at least about 5 amino acid residues, more preferably at least about 10, and most preferably at least about 20 amino acid residues. Functional portions of the inventive polypeptides may be identified by first preparing fragments of the polypeptide, by either chemical or enzymatic digestion of the polypeptide or mutation analysis of the polynucleotide that encodes for the polypeptide, and subsequently expressing the resultant mutant polypeptides. The polypeptide fragments or mutant polypeptides are then tested to determine which portions retain the biological activity of the full-length polypeptide. Portions and other variants of the inventive polypeptides may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain (Merrifield, *J. Am. Chem. Soc.* 85:2149-2154, 1963). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions. Variants of a native polypeptide may be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (see, for example, Kunkel, *Proc. Natl. Acad. Sci. USA* 82:488-492, 1985). Sections of DNA sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

The polynucleotide sequences of the present invention, derived from *fsn* -/- mouse lymph node stromal cells, encode polypeptides that have important role(s) in growth and

development of the immune system, and in responses of the immune system to tissue injury and inflammation as well as other disease states. Some of the polynucleotides contain sequences that code for signal sequences, or transmembrane domains, which identify the protein products as secreted molecules or receptors. Such polypeptide products include growth factors, cytokines, or their cognate receptors. The polypeptide sequence of SEQ ID NO: 13 has more than 25% identity to members of the tumour necrosis factor (TNF) receptor family of proteins; the polypeptides of SEQ ID NO: 30, 31, 32 and 33 have more than 25% identity to members of the fibroblast growth factor (FGF) receptor family of proteins; and the polypeptide of SEQ ID NO: 38 has more than 25% identity to members of the WDNM1 family of proteins. These identified polypeptides have similar biological functions.

In particular, the inventive polypeptides have important roles in processes such as: modulation of immune responses; differentiation of precursor immune cells into specialized cell types; cell migration; cell proliferation and cell-cell interaction. The polypeptides are important in the defence of the body against infectious agents, and thus important in maintaining a disease-free environment. These polypeptides act as modulators of skin cells, especially since immune cells infiltrate skin during tissue insult, causing growth and differentiation of skin cells. In addition, these polypeptides are immunologically active, making them important therapeutic targets in a large range of disease states.

In one aspect, the present invention provides methods for using one or more of the inventive polypeptides or polynucleotides to treat disorders in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human.

In this aspect, the polypeptide or polynucleotide is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines may comprise one or more of the above polypeptides and a non-specific immune response amplifier, such as an adjuvant or a liposome, into which the polypeptide is incorporated.

Alternatively, a vaccine or pharmaceutical composition of the present invention may contain DNA encoding one or more polypeptides as described above, such that the polypeptide is generated *in situ*. In such vaccines and pharmaceutical compositions, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, and bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminator signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus Calmette-Guerin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other poxvirus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic, or defective, replication competent virus. Techniques for incorporating DNA into such expression systems are well known in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al.*, *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

Routes and frequency of administration, as well as dosage, vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intradermal, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg per kg of host, and preferably from about 100 pg to about 1 μg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 ml to about 2 ml.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a lipid, a

wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of adjuvants may be employed in the vaccines derived from this invention to non-specifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a non-specific stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *M. tuberculosis*. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories, Detroit, MI), and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ). Other suitable adjuvants include alum, biodegradable microspheres, monophosphoryl lipid A and Quil A.

The polynucleotides of the present invention may also be used as markers for tissue, as chromosome markers or tags, in the identification of genetic disorders, and for the design of oligonucleotides for examination of expression patterns using techniques well known in the art, such as the microarray technology available from Synteni (Palo Alto, CA). Partial polynucleotide sequences disclosed herein may be employed to obtain full length genes by, for example, screening of DNA expression libraries, and to isolate homologous DNA sequences from other species using hybridization probes or PCR primers based on the inventive sequences.

The isolated polynucleotides of the present invention also have utility in genome mapping, in physical mapping, and in positional cloning of genes. As detailed below, the polynucleotide sequences identified as SEQ ID NO: 1-10, 21-29, 39-46 and 58, and their variants, may be used to design oligonucleotide probes and primers. Oligonucleotide probes designed using the polynucleotides of the present invention may be used to detect the presence and examine the expression patterns of genes in any organism having

sufficiently similar DNA and RNA sequences in their cells using techniques that are well known in the art, such as slot blot DNA hybridization techniques. Oligonucleotide primers designed using the polynucleotides of the present invention may be used for PCR amplifications. Oligonucleotide probes and primers designed using the polynucleotides of the present invention may also be used in connection with various microarray technologies, including the microarray technology of Synteni (Palo Alto, California).

As used herein, the term "oligonucleotide" refers to a relatively short segment of a polynucleotide sequence, generally comprising between 6 and 60 nucleotides, and comprehends both probes for use in hybridization assays and primers for use in the amplification of DNA by polymerase chain reaction. An oligonucleotide probe or primer is described as "corresponding to" a polynucleotide of the present invention, including one of the sequences set out as SEQ ID NO: 1-10, 21-29 and 39-46, or a variant thereof, if the oligonucleotide probe or primer, or its complement, is contained within one of the sequences set out as SEQ ID NO: 1-10, 21-29, 39-46 and 58, or a variant of one of the specified sequences. Oligonucleotide probes and primers of the present invention are substantially complementary to a polynucleotide disclosed herein.

Two single stranded sequences are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared, with the appropriate nucleotide insertions and/or deletions, pair with at least 80%, preferably at least 90% to 95% and more preferably at least 98% to 100% of the nucleotides of the other strand. Alternatively, substantial complementarity exists when a first DNA strand will selectively hybridize to a second DNA strand under stringent hybridization conditions. Stringent hybridization conditions for determining complementarity include salt conditions of less than about 1 M, more usually less than about 500 mM, and preferably less than about 200 mM. Hybridization temperatures can be as low as 5°C, but are generally greater than about 22°C, more preferably greater than about 30°C, and most preferably greater than about 37°C. Longer DNA fragments may require higher hybridization temperatures for specific hybridization. Since the stringency of hybridization may be affected by other factors such as probe composition, presence of

organic solvents and extent of base mismatching, the combination of parameters is more important than the absolute measure of any one alone.

In specific embodiments, the oligonucleotide probes and/or primers comprise at least about 6 contiguous residues, more preferably at least about 10 contiguous residues, and most preferably at least about 20 contiguous residues complementary to a polynucleotide sequence of the present invention. Probes and primers of the present invention may be from about 8 to 100 base pairs in length or, preferably from about 10 to 50 base pairs in length or, more preferably from about 15 to 40 base pairs in length. The probes can be easily selected using procedures well known in the art, taking into account DNA-DNA hybridization stringencies, annealing and melting temperatures, and potential for formation of loops and other factors, which are well known in the art. Tools and software suitable for designing probes, and especially suitable for designing PCR primers, are available on the Internet, for example, at URL http://www.horizonpress.com/pcr/. A software program suitable for designing probes, and especially for designing PCR primers, is available from Premier Biosoft International, 3786 Corina Way, Palo Alto, CA 94303-4504. Preferred techniques for designing PCR primers are also disclosed in Dieffenbach, CW and Dyksler, GS. *PCR Primer: a laboratory manual*, CSHL Press: Cold Spring Harbor, NY, 1995.

A plurality of oligonucleotide probes or primers corresponding to a polynucleotide of the present invention may be provided in a kit form. Such kits generally comprise multiple DNA or oligonucleotide probes, each probe being specific for a polynucleotide sequence. Kits of the present invention may comprise one or more probes or primers corresponding to a polynucleotide of the present invention, including a polynucleotide sequence identified in SEQ ID NO: 1-10, 21-29, 39-46 and 58.

In one embodiment useful for high-throughput assays, the oligonucleotide probe kits of the present invention comprise multiple probes in an array format, wherein each probe is immobilized at a predefined, spatially addressable location on the surface of a solid substrate. Array formats which may be usefully employed in the present invention are disclosed, for example, in U.S. Patents No. 5,412,087 and 5,545,451, and PCT

Publication No. WO 95/00450, the disclosures of which are hereby incorporated by reference.

The polynucleotides of the present invention may also be used to tag or identify an organism or reproductive material therefrom. Such tagging may be accomplished, for example, by stably introducing a non-disruptive non-functional heterologous polynucleotide identifier into an organism, the polynucleotide comprising one of the polynucleotides of the present invention.

The polypeptides provided by the present invention may additionally be used in assays to determine biological activity, to raise antibodies, to isolate corresponding ligands or receptors, in assays to quantify levels of protein or cognate corresponding ligand or receptor, as anti-inflammatory agents, and in compositions for the treatment of diseases of skin, connective tissue and the immune system.

Example 1

ISOLATION OF CDNA SEQUENCES FROM LYMPH NODE STROMAL CELL EXPRESSION LIBRARIES

The cDNA sequences of the present invention were obtained by high-throughput sequencing of cDNA expression libraries constructed from rodent *fsn* -/- lymph node stromal cells as described below.

cDNA Libraries from Lymph Node Stromal Cells (MLSA and MLSE)

Lymph nodes were removed from flaky skin *fsn* -/- mice, the cells dissociated and the resulting single cell suspension placed in culture. After four passages, the cells were harvested. Total RNA, isolated using TRIzol Reagent (BRL Life Technologies, Gaithersburg, MD), was used to obtain mRNA using a Poly(A) Quik mRNA isolation kit (Stratagene, La Jolla, CA), according to the manufacturer's specifications. A cDNA expression library (referred to as the MLSA library) was then prepared from the mRNA by Reverse Transcriptase synthesis using a Lambda ZAP Express cDNA library synthesis kit (Stratagene, La Jolla, CA). A second cDNA expression library, referred to as the

MLSE library, was prepared exactly as above except that the cDNA was inserted into the mammalian expression vector pcDNA3 (Invitrogen, Carlsbad CA).

The nucleotide sequence of the cDNA clone isolated from the MLSE library is given in SEQ ID NO: 1, with the corresponding amino acid sequence being provided in SEQ ID NO: 11. The nucleotide sequences of the cDNA clones isolated from the MLSA library are given in SEQ ID NO: 2-10, 21-23 and 28, with the corresponding amino acid sequences being provided in SEQ ID NO: 12-20, 30-32 and 37, respectively.

Subtracted cDNA Library from flaky skin Lymph Node Stromal Cells (MLSS)

Stromal cells from flaky skin mice lymph nodes and 3T3 fibroblasts were grown in culture and the total RNA extracted from these cells using established protocols. Total RNA from both populations was isolated using TRIzol Reagent (Gibco BRL Life Technologies, Gaitherburg, MD) and used to obtain mRNA using either a Poly (A) Quik mRNA isolation kit (Stratagene, La Jolla, CA) or Quick Prep(R) Micro mRNA purification kit (Pharmacia, Uppsala, Sweden). Double-stranded cDNA from flaky skin lymph node stromal cell mRNA was prepared by Reverse Transcriptase synthesis using a lambda ZAP cDNA library synthesis kit (Stratagene) that had been ligated with *Eco*RI adaptors and digested with *Xho*I to produce double-stranded fragments with *Eco*RI and *Xho*I overhanging ends.

Double-stranded cDNA from 3T3 fibroblasts was prepared using the Superscript II reverse transcriptase (Gibco BRL Life Technologies) followed by treatment with DNA polymerase I and RNaseH (Gibco BRL Life Technologies). Double-stranded 3T3 cDNA was then digested with restriction endonucleases *Alu*I and *Rsa*I (Gibco BRL Life Technologies) to produce blunt-ended fragments. A 20-fold excess of *Alu*I /*Rsa*I-digested 3T3 cDNA was hybridized with the *Eco*RI/*Xho*I flaky skin lymph node stromal cell cDNA in the following hybridisation solution: 50% formamide, 5xSSC, 10mM NaH_2PO_4 pH7.5, 1mM EDTA, 0.1% SDS, 200µg yeast tRNA (Boehringer Mannheim) at 37°C for 24 hours. Hybridized flaky skin lymph node stromal cell cDNA and 3T3 cDNA was then phenol/chloroform extracted and ethanol precipitated. The cDNA was size-fractionated

over a Sepharose CL-2B gel filtration column as described in the Lambda ZAP cDNA library synthesis protocol (Stratagene). Flaky skin lymph node stromal cell-specific cDNA was preferentially ligated into ZAP Express vector (Stratagene) by virtue of *Eco*RI/*Xho*I ends. Chimeric cDNA between flaky skin lymph node stromal cell cDNA and 3T3 cDNA would not be cloned due to non-compatible ends, and the subtracted cDNA library was packaged using Gigapack III Gold packaging extract (Stratagene).

The nucleotide sequences of the cDNA clones isolated from the MLSS library are given in SEQ ID NO: 25-27 and 29, with the corresponding amino acid sequences being provided in SEQ ID NO: 34-36 and 38, respectively.

Example 2

CHARACTERIZATION OF ISOLATED cDNA SEQUENCES

The isolated cDNA sequences were compared to sequences in the EMBL DNA database using the computer algorithm BLASTN, and the corresponding polypeptide sequences (DNA translated to protein in each of 6 reading frames) were compared to sequences in the SwissProt database using the computer algorithm BLASTP. Specifically, comparisons of DNA sequences provided in SEQ ID NO: 1-10, 21-29 and 39-46 to sequences in the EMBL (Release 60, September 1999) DNA database, and amino acid sequences provided in SEQ ID NO: 11-20, 30-38 and 47-53 to sequences in the SwissProt and TrEMBL (up to October 20, 1999) databases were made as of December 31, 1999. Comparisons of DNA sequences provided in SEQ ID NO: 58 to sequences in the EMBL (Release 62, April 2000) DNA database, and amino acid sequences provided in SEQ ID NO: 59 to sequences in the SwissProt and TrEMBL (up to April 7, 2000) databases were made as of July 11, 2000. The cDNA sequences of SEQ ID NO: 1-10, 21-24 and 27-28, and their corresponding polypeptide sequences (SEQ ID NO: 11-20, 30-33 and 36-37, respectively) were determined to have less than 75% identity (determined as described above) to sequences in the EMBL and SwissProt databases using the computer algorithms BLASTN and BLASTP, respectively. The polypeptide of

SEQ ID NO: 59 was determined to have less than 75% identity (determined as described above) to sequences in the SwissProt database using the computer algorithm BLASTP.

Isolated cDNA sequences and their corresponding polypeptide sequences, were computer analyzed for the presence of signal sequences identifying secreted molecules. Isolated cDNA sequences that have a signal sequence at a putative start site within the sequence are provided in SEQ ID NO: 4-6, 9-10, 25-26, 39-41 and 43-45. The isolated cDNA sequences were also computer analyzed for the presence of transmembrane domains coding for putative membrane-bound molecules. Isolated cDNA sequences that have one or more transmembrane domain(s) within the sequence are provided in SEQ ID NO: 1-3, 7, 8, 27 and 41-45.

Using automated search programs to screen against sequences coding for known molecules reported to be of therapeutic and/or diagnostic use, the isolated polynucleotides of SEQ ID NO: 3, 21-24 and 29 were determined to encode polypeptide sequences that are members of the tumour necrosis factor (TNF) receptor family of proteins (SEQ ID NO: 13), the fibroblast growth factor (FGF) receptor family (SEQ ID NO: 30-33), the WDNM1 protein family (SEQ ID NO: 38) and the serine/threonine protein kinase family (SEQ ID NO: 59). A family member is here defined to have at least 20% identical amino acid residues in the translated polypeptide to a known protein or member of a protein family.

As noted above, the isolated cDNA sequence of SEQ ID NO: 3 was determined to encode a polypeptide (SEQ ID NO: 13) that is a member of the TNF-receptor family. Proteins of the TNF/NGF-receptor family are involved in the proliferation, differentiation and death of many cell types including B and T lymphocytes. Residues 18-55 of SEQ ID NO: 13 show a high degree of similarity to the Prosite motif for the TNF/NGF receptor family (Banner *et al.*, *Cell* 73:431-445, 1993). This motif contributes to the ligand binding domain of the molecule and is thus essential to its function. (Gruss and Dower, *Blood* 85:3378-3404, 1995). The polypeptide of SEQ ID NO: 13 therefore influences the growth, differentiation and activation of several cell types, and has utility as an agent for

the treatment of skin wounds, and the treatment and diagnosis of cancers, inflammatory diseases, and growth and developmental defects.

The isolated cDNA sequence of SEQ ID NO: 29 was determined to encode a polypeptide (SEQ ID NO: 38) that is a member of the WDNM1 protein family. The WDNM1 family of proteins has a conserved arrangement of cysteine residues. The family includes several proteinase inhibitors, indicating that WDNM1 encodes a product with proteinase inhibiting capacity. The WDNM1 gene has been shown to be down-regulated in metastatic rat mammary adenocarcinomas (Dear and Kefford, *Biochem. Biophys. Res. Comm.* 176:247-254, 1991).

The isolated cDNA sequence of SEQ ID NO: 21 was determined to encode a protein sequence (SEQ ID NO: 30) that is a member of the fibroblast growth factor (FGF) receptor family of proteins, specifically the FGF receptor 3. Fibroblast growth factor receptors belong to a family of four single membrane-spanning tyrosine kinases (FGFR1 to 4). These receptors serve as high-affinity receptors for 17 growth factors (FGF1 to 17). FGF receptors have important roles in multiple biological processes, including mesoderm induction and patterning, cell growth and migration, organ formation and bone growth (Xu, *Cell Tissue Res.* 296:33-43, 1999). Further analysis of the sequence revealed the presence of a putative transmembrane domain and intracellular domain, similar to other FGF receptors.

The isolated cDNA sequence of SEQ ID NO: 44 was determined to encode a polypeptide (SEQ ID NO: 52) corresponding to a lysyl oxidase-related protein. Lysyl oxidase is a copper-dependent amine oxidase that has an important role in the formation of connective tissue matrices. The molecule is involved in crosslinking of the extracellular matrix proteins, collagen and elastin (Smith-Mungo and Kagan, *Matrix Biol.* 16:387-398, 1998). Expression of lysyl oxidase is upregulated in many fibrotic diseases, and down regulated in diseases involving impaired copper metabolism. Identification of new lysyl oxidase-related proteins indicates the existence of a multigene family. Experimental evidence suggests that lysyl oxidase may have other important biological

functions in addition to its role in cross-linking of collagen and elastin (Smith-Mungo and Kagan, *Matrix Biol.* 16:387-398, 1998).

The isolated cDNA sequence of SEQ ID NO: 45 was determined to encode a polypeptide (SEQ ID NO: 53) of a CD99-like protein. CD99, also referred to as MIC2, is a cell surface molecule involved in T cell adhesion processes (Gelin *et al.*, *EMBO J.* 8:3252-3259).

The isolated cDNA sequence of SEQ ID NO: 58 was determined to encode a polypeptide sequence (SEQ ID NO: 59) that corresponds to a serine/threonine protein kinase. Serine/threonine kinases participate in cell cycle progression and signal transduction. They are involved in mediating intracellular responses to external signals, such as growth factors, hormones and neurotransmitters, and are involved in cell proliferation and oncogenesis.

Example 3

ISOLATION OF FULL LENGTH cDNA SEQUENCE OF A MURINE FIBROBLAST GROWTH FACTOR RECEPTOR HOMOLOG

The full-length cDNA sequence of a murine fibroblast growth factor receptor homolog was isolated as follows.

The MLSA cell cDNA library (described in Example 1) was screened with an [α ^{32}P]-dCTP labeled cDNA probe corresponding to nucleotides 1 to 451 of the coding region within SEQ ID NO: 21. Plaque lifts, hybridization and screening were performed using standard molecular biology techniques. The determined polynucleotide sequence of the full-length murine FGFR gene (referred to as muFGFR-β) is provided in SEQ ID NO: 22, with the corresponding polypeptide sequence being provided in SEQ ID NO: 31.

Analysis of the polynucleotide sequence of SEQ ID NO: 22 revealed the presence of a putative transmembrane domain corresponding to nucleotides 1311 to 1370. The polypeptide sequence (SEQ ID NO: 31; Figure 1) has regions similar to the extracellular domain of the fibroblast growth factor receptor family. The amino acid sequence of the

extracellular domain of muFGFR-β is provided in SEQ ID NO: 60, while the amino acid sequence of the intracellular domain is provided in SEQ ID NO: 61.

A splice variant of SEQ ID NO: 22 was also isolated from the MLSA cDNA library as described in Example 1. The determined polynucleotide sequence of the splice variant (referred to as FGFR-γ) is provided in SEQ ID NO: 23 and the corresponding polypeptide sequence is provided in SEQ ID NO: 32. The splice regions are in an equivalent position to splice sites for previously described FGF receptors (Ornitz, *J. Biol. Chem.* 296:15292-15297, 1996; Wilkie, *Current Biology* 5:500-507, 1995; Miki, *Proc. Natl. Acad. Sci. USA* 89:246-250, 1992), thus establishing that this molecule (referred to as FGFR5) is a FGF receptor homolog. The main difference between the two FGFR5 splice variants is that muFGFR-β contains three extracellular Ig-domains, while FGFR-γ contains only two such domains.

To examine the structural similarities between FGFR5 and the other members of the FGF receptor family, 3D Swiss modeller (Petisch, *Bio/Technology* 13:658-660, 1995; Peitsch, *Biochem Soc Trans.* 24:274-279, 1996; Guex and Peitsch, *Electrophoresis* 18:2714-2723, 1997) was employed to produce a predicted crystal structure of the extracellular domain of FGFR-γ. These studies showed that the crystal structure of FGFR5 deviates from that of the known FGFR1 structure between residues 188 and 219 of SEQ ID NO: 32. These residues correlate with an area of low homology between FGFR5 and other members of the FGF receptor family that may have a critical role in defining ligand specificity.

The critical residues for ligand binding have previously been identified in co-crystallization studies of FGFR1 binding FGF-2 (Plotnikov et al., *Cell* 98:641-650, 1999). Alignment of FGFR-γ with FGFR1 showed that many of these residues are conserved or are a conservative substitution. Conserved ligand binding residues between the two receptors are found at residues 66, 68, 146, 178, 181, 183 and 216 of SEQ ID NO: 32, while conservative substitutions of potential ligand binding residues are found at residues 64, 180 and 226 of SEQ ID NO: 32. When visualized on the predicted crystal structure of FGFR-γ, these residues line the groove of the ligand binding domain. Thus,

while the overall degree of similarity between FGFR5 and other FGF receptors is relatively low, the extracellular domains of the two FGFR5 splice variants have all the conserved residues important for ligand binding.

The main difference between the FGFR5 receptor and other family members is the lack of an intracellular tyrosine kinase domain. With the four previously identified FGF receptors (FGFR1-4), signal transduction is mediated by ligand binding and receptor dimerization, resulting in autophosphorylation of the tyrosine residues within the intracellular RTK domain. This autophosphorylation then phosphorylates a number of intracellular substrates, initiating several signal transduction cascades. The FGFR5 splice variants described herein each contain tyrosine residues in the intracellular domain demonstrating similarity to a SHP binding motif (residues 458-463 of SEQ ID NO: 31 and 367-377 of SEQ ID NO: 32). SHPs are protein tyrosine phosphatases that participate in cellular signalling and that have previously been identified in the cytoplasmic domains of many receptors eliciting a broad range of activities. The presence of such motifs in the cytoplasmic domain of FGFR5 is thus indicative of signalling, and modification of these motifs may be employed to modulate signal transduction initiated by binding of a ligand to FGFR5. These motifs are conserved between the mouse transcripts of FGFR5 and the human homolog described below.

Example 4

ISOLATION OF A HUMAN FGF RECEPTOR HOMOLOG

The cDNA EST encoding the partial murine FGF receptor (SEQ ID NO: 21) was used to search the EMBL database (Release 58, March 1999) to identify human EST homologs. The identified EST (Accession Number AI245701) was obtained from Research Genetics, Inc (Huntsville AL) as I.M.A.G.E. Consortium clone ID 1870593. Sequence determination of the complete insert of clone 1870593 resulted in the identification of 520 additional nucleotides. The insert of this clone did not represent the full-length gene. The determined nucleotide sequence of the complete insert of clone

1870593 is given in SEQ ID NO: 24 and the corresponding polypeptide sequence in SEQ ID NO: 33.

Example 5

CHARACTERIZATION OF MURINE FGF RECEPTOR HOMOLOG

Soluble forms of the murine FGF receptor homolog, muFGFR-β and splice variant FGFR-γ (SEQ ID NO: 22 and 23, respectively) were expressed in mammalian cells and the purified proteins used to determine the ligand binding specificity of the molecules as follows.

The extracellular domains of muFGFR-β and FGFR-γ were amplified by PCR using primers MS158 and MS159 (SEQ ID NO: 55 and 56, respectively) and cloned into the expression vector pcDNA3 containing the Fc fragment from human IgG1. These soluble recombinant proteins, referred to as FGFRβFc and FGFRγFc, were expressed in HEK293 cells (ATCC No. CRL-1573, American Type Culture Collection, Manassas, VA) and purified using an Affiprep protein A column (Biorad, Hercules CA).

FGF-2 (basic fibroblast growth factor) has previously been demonstrated to bind all FGF receptors but with a range of different affinities. Binding of muFGFR-β to FGF-2 was demonstrated by co-incubating the purified protein and FGF-2 in the presence of protein G Sepharose (Amersham Pharmacia, Uppsala, Sweden) and resolving complexes formed on denaturing polyacrylamide gels. FGF-2 (2 μg) was incubated with 5 μg FGFRβFc, FGF Receptor 2 (FGFR2Fc) or unrelated protein (MLSA8790Fc) in 5 μl protein G fast flow beads (Pharmacia, Uppsala, Sweden), PBS and 0.1% Triton X-100 for 60 min at 4°C. The beads were washed three times in 0.1% Triton X-100/PBS and resuspended in 20 μl loading buffer (0.1 M DTT, 10% sucrose, 60 mM Tris.HCl pH 6.8, 5% SDS and 0.01% bromophenol blue). The samples were analysed on a 12% polyacrylamide gel. FGF-2, FGFR2Fc, FGFRβFc and MLSA8790Fc (1 μg of each) were loaded on the gel for comparison. After staining of the gel with Coomassie blue, a doublet of bands were visible in the lane containing FGFRβFc, indicating that a complex

formed between the FGF-2 and the murine FGF receptor homolog FGFRβFc, and that FGF-2 is a ligand for the novel FGF receptor homolog. A doublet was also observed in the lane containing the FGFR2Fc, which was the positive control. No doublet was observed in the negative control lane containing the MLSA8790Fc protein.

The binding specificity of the murine FGF receptor homolog FGFRβFc was further examined by repeating the experiment described above, replacing the FGF-2 with another known growth factor, epidermal growth factor (EGF). In this experiment, EGF did not bind to FGFR2Fc, FGFRβFc or MLSA8790Fc, indicating that binding of FGF-2 to the murine FGF receptor homolog FGFRβFc was specific. Similarly, in subsequent experiments employing FGF-7, no binding of FGFR2Fc, FGFRβFc or MLSA8790Fc was observed.

To determine the difference in binding affinity between FGFR5 and FGFR2, the ability of FGFRβFc and FGFRγFc to inhibit FGF signalling in FGF-responsive NIH-3T3 SRE reporter cells was examined. Fibroblast growth factors typically signal via phosphorylation of the receptor tyrosine kinase domain stimulating the MAP kinase pathway. This eventually leads to activation of genes under the control of the serum response element (SRE). Reporter constructs containing concatamerised SRE sequences upstream of a luciferase reporter gene were stably transfected into NIH-3T3 cells. Reporter activity was measured by measuring luciferase levels. As shown in Figure 2A, a dose dependent response of NIH-3T3 SRE cells to FGF-2 was seen in the presence of heparin. Using a standard dose of FGF-2 in the presence of heparin, an increasing concentration of FGFR2Fc, FGFRβFc or FGFRγFc was titrated onto the NIH-3T3 SRE cells and luciferase activity was measured. Increasing concentrations of FGFR2Fc, the positive control, reduced the luciferase signal in FGF-2 stimulated cells (Figure 2B). However, titrating FGFRβFc and FGFRγFc did not inhibit FGF-mediated luciferase signal from the NIH-3T3 SRE cells. These results show that FGF-2 has lower affinity for either FGFRβ or FGFRγ than for FGFR2, and indicate that the ligand specificity of FGFR5 is different to those of the other members of the FGF receptor family.

Example 6

SEQUENCE DETERMINATION OF A POLYNUCLEOTIDE FRAGMENT CONTAINING GENOMIC MURINE FGFRβ

As noted above, the two splice variants muFGFR-β and FGFR-γ do not contain the classical receptor tyrosine kinase domain present in other known FGF receptors. In order to investigate whether FGFR5 contains a splice variant with a classical receptor tyrosine kinase (RTK) domain, the genomic DNA of FGFR5 was sequenced as follows.

Mouse genomic DNA was isolated from L929 cells using standard techniques. A genomic polynucleotide fragment containing murine FGFRβ was PCR amplified using primers MS157 and MS166 (SEQ ID NO: 56 and 57, respectively). The 1.4 kb polynucleotide fragment was cloned into a T-tailed pBluescript SK^{2+} vector. The sequence of the insert of this plasmid was determined using standard primer walking sequencing techniques. The sequence of the genomic fragment containing murine FGFRβ is given in SEQ ID NO: 46. This sequence extends from the 3' untranslated region to the end of the mature FGFR5 receptor minus the signal sequence. No alternative exons containing an RTK domain were identified.

Example 7

STIMULATION OF CELL GROWTH BY MURINE FGFR5β AND FGFR5γ

The stimulation of RAW264.10 cells (Hamilton *et al., J. Exp. Med.* 148:811-816, 1978) and peripheral blood mononuclear cells (PBMC) in the presence of the murine FGFRβ and FGFRγ (also referred to herein as FGFR5β and FGFR5γ, respectively) was demonstrated as follows. RAW264.10 cells are derived from a murine macrophage cell line generated from BALB/c mice, and are macrophage and osteoblast precursors.

The murine FGF receptor homolog, muFGFRβ, and splice variant FGFRγ (SEQ ID NO: 22 and 23, respectively) were expressed in mammalian cells and purified as murine FGFR5β Fc fusion protein and FGFR5γ Fc fusion protein as described above.

The FGFR5β and FGFR5γ Fc fusion proteins were titrated from 10 nM in 0.05 ml media (DMEM supplemented with 5%FBS, 2mM L-glutamine (Sigma, St Louis MO), 1mM sodium pyruvate (Life Technologies, Gibco BRL, Gaithersburg MD), 0.77mM L-asparagine (Sigma), 0.2mM arginine (Sigma), 160mM penicillin G (Sigma), 70mM dihydrostreptomycin sulfate (Boehringer Mannheim, Roche Molecular Biochemicals, Basel, Switzerland) in a 96 well flat-bottomed microtitre plate. Purified human FGFR2 Fc fusion protein was used as control and titrated from 10 nM. RAW264.10 cells were added to each well in 0.05 ml media at a concentration of 2×10^4 cells/ml. The plate was incubated at 37°C in a humidified atmosphere containing 10% CO_2 for 4 days. Cell growth was determined by MTS dye conversion and quantified using an ELISA reader. As shown in Figure 3, both murine FGFR5β and FGFR5γ Fc fusion proteins stimulated the growth of RAW264.10 cells at concentrations of 100 pM and greater.

Purified FGFR5β and FGFR5γ Fc fusion proteins were titrated from 20 nM into 0.1 ml media per well of 96 well microtiter plates. Purified human FGFR2 Fc fusion protein and human IgG Fc were used as controls. PBMC were harvested from blood by density gradient centrifugation and resuspended in media to a concentration of 2×10^6 cells/ml. Phytokemagglutinin (PHA), Pokeweed mitogen (PWM), anti-CD3 antibody or media was added to the PBMC and 0.1ml of cells dispensed to each well. The plates were incubated for 3 days at 37°C in a humidified atmosphere containing 5% CO_2 in air. Cell proliferation was quantified by pulsing the plates with tritiated thymidine for the final 16 hours of culture. The cells were then harvested and tritiated thymidine incorporation enumerated by standard liquid scintillation counting. Figures 4-6 show that murine FGFR5β and FGFR5γ fusion proteins enhanced the proliferation of PBMC activated with either PHA or anti-CD3 but did not induce the proliferation of PBMC on their own. Stimulation of proliferation was not observed with human FGFR2 Fc fusion protein or human IgG Fc.

These results demonstrate that FGFR5β and FGFR5γ are immunostimulatory molecules that directly activate a macrophage cell line. The macrophage cell line used in these assays (RAW264.10) has previously been shown to differentiate into osteoblasts

when stimulated with a variety of known bone morphogenic agents. The effects of FGFR5β and FGFR5γ on these cells suggest that these molecules may also stimulate the differentiation and activation of osteoblasts. Weidemann and Trueb (*Genomics 69*:275-279, 2000), have shown that FGFR5 is expressed in cartilaginous tissues. When combined with the data provided above, this suggests that FGFR5 may play a role in bone formation and may therefore have applications in fracture repair and bone diseases, such as osteoporosis and osteopetrosis.

Example 8

STIMULATION OF PROLIFERATION OF ADHERENT PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) BY MURINE FGFR5β AND FGFR5γ

Stimulation of PBMC to adhere to plastic by murine FGFR5β and FGFR5γ Fc fusion proteins was demonstrated as follows.

MuFGFR5β and muFGFR5γ (SEQ ID NO: 22 and 23, respectively) were expressed in mammalian cells and purified as Fc fusion proteins as described above. The muFGFR5β and muFGFR5γ Fc fusion proteins were titrated from 10 nM into 0.1 ml media per well of 96 well microtitre plates. Peripheral blood mononuclear cells (PBMC) were harvested from blood by density gradient centrifugation and resuspended in media to a concentration of 2 x 10^6 cells/ml. PHA or media (RPMI 1640 supplemented with 5% FBS, 2mM L-glutamine (Sigma), 160mM penicillin G (Sigma), and 70mM dihydrostreptomycin sulfate (Boehringer Mannheim) was added to the PBMC and 0.1 ml of cells dispensed to each well. The plates were incubated for 3 days at 37°C in a humidified atmosphere containing 5% CO_2 in air. The non-adherent cells were removed with three media washes. Media (0.05 ml) containing MTS/PES solution (CellTiter96 Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI) was dispensed to each well and the plate incubated for 4 hrs before the degree of dye conversion was quantified using a 96 well ELISA reader. Figures 7 and 8 show that muFGFR5β and muFGFR5γ Fc fusion proteins stimulated the adherence and proliferation of adherent

PBMC in a dose dependent manner and that PHA stimulation augmented this effect. These results demonstrate that FGFR5β and FGFR5γ are able to enhance the proliferative effects of known immunostimulatory molecules on a mixed population of human haemopoietic cells, namely PBMC.

Example 9

ACTIVATION OF NATURAL KILLER CELLS BY MURINE FGFR5β AND FGFR5γ

Activation of Natural Killer (NK) cells by muFGFR5β and muFGFR5γ Fc fusion proteins was demonstrated as follows.

Peripheral blood mononuclear cells (PBMC) were harvested from blood by density gradient centrifugation and resuspended in media (RPMI 1640 supplemented with 5%FBS, 2mM L-glutamine (Sigma), 160mM penicillin G (Sigma), 70mM dihydrostreptomycin sulfate (Boehringer Mannheim)) to a concentration of 2 x 10^6 cells/ml. Purified muFGFR5β and muFGFR5γ Fc fusion protein were added to the cells at a concentration of 10 nM and the cells were cultured in 6 well plates (3 ml/well) for 3 days at 37°C in a humidified atmosphere containing 5% CO_2 in air. Purified human FGFR2 Fc fusion protein was used as control. The non-adherent cells were removed with three media washes. The adherent cells were collected by light trypsinization and scraping. The cells were washed into staining buffer and their phenotype determined by standard flow cytometric techniques using NK cell marker CD56 and a control isotype antibody.

As shown in Figure 9, muFGFR5β and muFGFR5γ Fc fusion proteins stimulated the adherence and/or growth of adherent cells from human PBMC, with approximately 50% of these cells being NK cells. The filled histograms represent the adherent PBMC stained with the NK cell marker CD56 and the open histograms represent the same cells stained with the isotype-matched control antibody. FGFR2 did not stimulate the adherence of PBMC and therefore there were no cells to analyze from these cultures. These results demonstrate that FGFR5β and FGFR5γ are immunostimulatory molecules

that directly activate NK cells. These results, plus those provided in Example 8, above, show that FGFR5 can enhance immune responses, and may thus be usefully employed to enhance vaccine responses and anti-cancer therapies.

Example 10

IMMUNOPRECIPITATION OF A 20-30 KDA SURFACE PROTEIN FROM RAW264.10 CELLS BY MuFGFR5β AND MuFGFR5γ FC FUSION PROTEINS

Immunoprecipitation of a 20-30 kDa protein from the surface of RAW264.10 cells by murine FGFR5β and FGFR5γ Fc fusion proteins, but not FGFR2 or control (murine MALA13003) Fc fusion proteins was demonstrated as follows. Murine MALA13003 is a novel receptor of unknown function.

Preparation of Protein A beads bound with murine FGFRβ and FGFR5γ Fc fusion proteins

Two µl Protein A Beads (Protein A Ceramic HyperDF, Gibco BRL) were dispensed into 4 microfuge tubes. The beads were washed three times in IP wash buffer (50mM Tris.HCl pH 7.5, 0.5 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% Tween-20). The IP wash buffer was removed and 20 µg purified murine FGFR5β, FGFR5γ , FGFR2 or murine MALA 31003 Fc fusion proteins was added to each microfuge tube and incubated on a rocking platform for either 2-3 hours at room temperature or overnight at 4°C. The beads were washed three times with IP wash buffer and centrifuged at 3,000 rpm for 2 min.

Preparation of cells

RAW264.10 cells were harvested when at 75% confluence by using single strength trypsin and washed in PBS. After counting, the cell concentration was adjusted to 5 x 10^6 cells/ml in 5 ml PBS.

Biotinylation

A stock solution of *N*-hydroxysuccinimido-biotin (NHS-Biotin, Sigma) at 10 mg/ml was prepared in PBS. An aliquot of this stock biotin solution was added immediately to the RAW264.10 cell suspension to a final concentration of 100 μg/ml biotin and mixed carefully. The cell suspension was incubated at room temperature for 60 min on a rocking platform to prevent the cells from settling. The cells were washed three times by centrifugation at 1,000 rpm for 7 min and resuspension in glycine buffer (PBS, 10 mM glycine, pH 7.5) to block all free NHS-Biotin sites. The glycine was removed by washing the cells twice in PBS. The cells were aliquoted into four 1 ml portions of 5 x 10^6 cells/ml each and pelleted by centrifugation.

Extraction of soluble proteins

One ml of extraction buffer (50mM Tris.HCl pH 7.5, 0.15 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1% NP40) was added to each cell pellet. Cells were disrupted by using a syringe until the cell pellet was solubilized. The solubilized cells were centrifuged at 10,000 rpm for 10 min at 4°C and the supernatant was collected.

Immunoprecipitation

The cell lysates were pre-cleared twice by adding 2 μl washed Protein A beads and incubating for 1 hour at room temperature. The cell suspension was centrifuged and the supernatant collected. One ml pre-cleared lysate was added to each of the microfuge tubes containing 2 μl protein A beads coated with FGFR5β, FGFR5γ, FGFR2 or MALA 31003 Fc fusion proteins and incubated overnight at 4°C on a rocking platform. The beads were washed four times in IP wash buffer with a centrifugation step (4,000 rpm for 2 min). After the first wash, the bead suspension was transferred to a new tube so that the final sample was not contaminated with non-bound recombinant protein that may be stuck to the side of the tube. The supernatant was aspirated leaving approximately 20 μl of liquid on the beads. After the final wash, all the remaining 20 μl of supernatant on the

beads was removed and the beads resuspended in 20 µl RSB (0.08 M Tris.HCl pH 6.8, 0.01 M DTT (Dithiothreitol), 2% SDS, 10% glycerol).

Polyacrylamide Gel Electrophoresis

The prepared beads were run on two resolving polyacrylamide gels following standard procedures. One gel was stained with Coomassie Blue and the second gel was blotted onto PVDF (polyvinylidene difluoride) membrane (Immobilon-P Transfer Membrane, Millipore, Bedford MA) by Western transfer following standard procedures. The blot was developed using ECL Western Blotting Detection Reagent (Amersham Pharmacia, Uppsala, Sweden) and exposed to Scientific Imaging film (Agfa Curix Blue HC-S Plus).

A protein of 20 to 30 kDa molecular weight was immunoprecipitated from the surface of RAW264.10 cells by the murine FGFR5β and FGFR5γ Fc fusion proteins, but not FGFR2 or MALA13003 Fc fusion proteins. This precipitated protein is likely to be the ligand or a component of the ligand complex recognized by FGFR5.

SEQ ID NOS: 1-61 are set out in the attached Sequence Listing. The codes for polynucleotide and polypeptide sequences used in the attached Sequence Listing conform to WIPO Standard ST.25 (1988), Appendix 2.

All references cited herein, including patent references and non-patent references, are hereby incorporated by reference in their entireties.

Although the present invention has been described in terms of specific embodiments, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.